# Description of a new branchiobdellidan species, with observations on three other species, and a key to the genus *Pterodrilus* (Annelida: Clitellata)

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Abstract.—Pterodrilus annulatus, a new species, is described from Tennessee, and compared with the west coast species Cambarincola serratus. An emended diagnosis of Pterodrilus and new observations on the anatomy of P. cedrus, P. choritonamus and P. hobbsi are presented. A key to the species of the genus, based on external segmental features, is given.

Information on the branchiobdellidan genus *Pterodrilus* was reviewed and extended by Holt (1968). The genus appears to be restricted to the eastern United States and its phylogenetic relationship with other genera was discussed in a synopsis of the Branchiobdellida (Holt 1986). More recently *Pterodrilus* was included in a checklist of North and Central American cambarincolids prepared by Holt & Opell (1993).

Collections of the crayfish, Orconectes placidens (Hagen, 1870), from a single site on the Harpeth River south of Franklin, Tennessee, yielded eight species of branchiobdellidans. Usually, at least six of these species were present on a single host. Live branchiobdellidans were relaxed in Perrier Water, fixed in formalin-ethanol-acetic acid (FAA), dehydrated in a graded series of ethanol:water solutions, cleared in methyl salicylate, and mounted on a microscope slide in Canada balsam (Brinkhurst & Gelder 1991). The anatomical nomenclature used in this paper follows that described in Brinkhurst & Gelder (1991) with two exceptions. The spermiducal gland and ejaculatory duct are now referred to as the glandular atrium and muscular atrium respectively so that the terminology becomes consistent with that used in the rest of the Clitellata. Specimens were examined from the collections of Perry C. Holt (PCH), the National Museum of Natural History, Smithsonian Institution (USNM) and the author (SRG).

During the process of identifying the branchiobdellidans from the Harpeth River, a new species of Pterodrilus was recognized. To confirm the new species status, type specimens of four species of Pterodrilus and Cambarincola serratus Holt, 1981 were borrowed from the National Museum and examined. These studies resulted in a clarification of the published descriptions of some of the anatomical features in certain pterodrilids, and the correction of some previously identified specimens. The keys constructed by Holt (1968) and Holt & Opell (1993) for identifying species of Pterodrilus utilize both external and internal anatomical features. However, following my examinations of the literature and specimens, a key was devised that used only external anatomical features. This key should greatly simplify the identification of both living and preserved specimens for biologists interested in the genus Pterodrilus.

#### Pterodrilus Moore, 1895

*References.*—Moore 1895:449–456, Goodnight 1940:58, Holt 1968:1, 3, 1986: 698. *Diagnosis.*—Single anterior nephridial pore; vasa deferentia entering glandular atrium entally; body less than 2.0 mm long; dorsal ridge across segment 8, other segments may have ridges and additional finger-like projections; dental formula 5/4; prostate gland arises from ectal to mid region of glandular atrium, and incompletely separated from it; penis muscular, protrusible; spermatheca present (emended by SRG).

## Pterodrilus annulatus, new species Fig. 1A-F, 3G

*Type specimens.*—Holotype, USNM 169996, and two paratypes, USNM 169997, and 169998 on *Orconectes placidens* (Hagen) removed from the Harpeth River, 6 miles SE of Franklin, Williamson Co., Tennessee (35°59'N, 86°32'W) by S. R. Gelder and R. O. Brinkhurst, 17 Oct 1990.

Diagnosis.-Body about 1.0 mm long, very prominent transverse ridges across the dorsal and lateral surfaces of segments 2 through 8, the last is fan-like, supernumerary muscles present, no dorsal segmental appendages; dorsal lip four slight rises; oral papillae 16; jaws small, lightly sclerotized, dorsal kidney-shaped, ventral quadrangular, dental formula 5/4; glandular atrium subcylindrical, length about 1/2 and width 1/4 diameter of segment; prostate gland length and diameter subequal to glandular atrium, differentiated, ental bulb absent; muscular atrium length about 1/2 glandular atrium; bursa ovoid, length about 1/3 segment diameter, penial sheath in ental 1/3 of bursa; spermatheca length about 1.2 times diameter of segment, thick-walled ectal duct about <sup>1</sup>/<sub>3</sub> total length, bulb oval to sacculate, ental process absent.

*Etymology.*—For the prominent dorsolateral ridges on the body segments.

Description.—Eleven fixed specimens average 1.05 mm in length and range from 0.90 to 1.23 mm long (Fig. 1A). Live, unstressed specimens measure about 1.50 mm long. The body is essentially rod-like with prominent transverse ridges across the dorsal and lateral surfaces of segments 2 through 8. All ridges contain supernumerary muscles (Fig. 1C, sm), with the posterior-most ridge being the largest, and fanlike. Segment 1 may occasionally show a pronounced transverse ridge. However, this is a fixation artifact caused by the head retracting into the segment. The head is greater in diameter than segment 1 and about equal to the posterior attachment disc.

The peristomium consists of two lips: the dorsal usually has four slight rises although the margin may appear smooth, while the ventral lip has a median emargination. There are 16 oral papillae around the mouth. The jaws are small,  $<V_6$  head diameter in width, with sharply pointed teeth and a dental formula of 5/4 (Fig. 1B). The dorsal jaw is kidney-shaped and the ventral jaw is quadrangular when seen in dorsoventral aspect. The pharynx has three sulci and the median is the largest.

The male reproductive organs in segment 6 extend about <sup>3</sup>/<sub>4</sub> of the way dorsad (Fig. 1C). The subcylindrical, glandular atrium is about 1/2 the diameter of the segment long, and about 1/4 wide (short and thick). The epithelial cells contain densely packed granules, and deferent lobes are absent. The prostate gland arises from ectal third of glandular atrium, the length and diameter are subequal to those of the glandular atrium. The ectal portion of the prostate gland contains granular cells and the remainder is lined with highly vacuolated, "differentiated" gland cells. An ental bulb is absent. The muscular atrium length is about 1/2 that of the glandular atrium, and enters the bursa. The bursa is generally ovoid, and about  $\frac{1}{3}$  the diameter of the segment in length. The penial sheath is formed from the ental <sup>1</sup>/<sub>3</sub> of the bursa and partially surrounds the subspherical penis. Although the penis has not been observed in the projected position, it is referred to as "protrusible" following the functional interpretation made by Holt (1986).

The spermatheca length is about 1.2



Fig. 1. *Pterodrilus annulatus*: A, Lateral view of holotype, scale bar = 100  $\mu$ m; B, Oblique lateral and dorsal views respectively of the dorsal and ventral jaws, scale bar = 10  $\mu$ m; C, Lateral view of the reproductive systems in segments 5 and 6 in the holotype, scale bar = 20  $\mu$ m; D, Lateral view of a curled paratype specimen, scale bar = 100  $\mu$ m; E and F, Lateral views of segment 5 in two paratype specimens to show the range in size of the spermatheca, scale bar = 25  $\mu$ m. Abbreviations: b, bursa; ga, glandular atrium; ma, muscular atrium; pg, prostate gland; sm, supernumerary muscles; spb, spermathecal bulb; spd, spermathecal duct; vd, vas deferens.

times the diameter of segment 5 (Fig. 1C). The thick-walled, ectal duct is about <sup>1</sup>/<sub>3</sub> the total length of the organ. The bulb is oval, frequently with a recognizable narrowing ental region, but an ental process is absent. *Variations.*—The transverse segmental ridges are less obvious when a specimen is fixed in the curled position (Fig. 1D). The shape of the spermathecal bulb ranged from a tubular shape, which happened to be reflexed and contained no spermatozoa (Fig. 1E), to a sacculate bulb that occupied most of the dorsal segment and was filled with spermatozoa (Fig. 1F). All other apparent variations could be accounted for by contraction of the specimen due to its state at the time of fixation.

Other branchiobdellidans on the host.— Ankyrodrilus legeaus Holt, 1965; Cambarincola chirocephala Ellis, 1919; C. fallax Hoffman, 1963; C. philadelphicus (Leidy, 1851); Oedipodrilus oedipus Holt, 1967; Pterodrilus hobbsi Holt, 1968; and Xironodrilus formosa Ellis, 1918.

*Remarks.*—Specimens of *P. annulatus* were observed on the dorsal and lateral surfaces of the carapace, abdomen, and the cephalothoracic appendages of the host. Usually specimens were observed in a cleft or by a bunch of setae supporting large populations of diatoms. *Pterodrilus annulatus* were seen to ingest these diatoms and they appear to form a significant portion of the diet based on the intestinal contents of fixed specimens.

Adjacent to the setal clumps and in the clefts of the exoskeleton, *P. annulatus* was often observed feeding alongside specimens of *P. hobbsi*. The two species showed no aggressive behavior towards one another.

This manuscript was originally submitted for publication in 1993. During the subsequent review period the author obtained a list of the branchiobdellidan species deposited in the National Museum. The collection contained a specimen (USNM 99811) with the unpublished, and therefore unrecognized name of Pterodrilus annulatus. This specimen, along with three others from two different species were mounted on the same slide. These branchiobdellidans were subsequently remounted on separate slides. All of these specimen were from lot USNM 99807 (1-769), "Unidentified Branchiobdellids," a collection removed from Orconectes neglectus neglectus and Orconectes ozarkensis by Perry C. Holt on 7 July 1958, obtained at Wildcat Creek adjacent to U.S. Route 68, Benton County, Arkansas (Worm General Catalog, National Museum of Natural History, Smithsonian Institution, Washington, DC). Two specimens of *P. annulatus* were obtained from this lot and mounted for species confirmation (USNM 169999, USNM 170000). All three branchiobdellidans were of the same species as that already described by the author. To avoid future problems, the author withdrew his manuscript and changes the name of his new species to *P. annulatus*.

Pterodrilus annulatus has an external appearance and size very similar to Cambarincola serratus Holt, 1981, reported from Idaho. However, C. serratus differs from P. annulatus in that the former has a dental formula of 5/5, the differentiated prostate gland arises from the glandular atrium very close to the muscular atrium entrance, the penis fills over two-thirds of the bursal atrium, and the long spermatheca has a sub-spherical terminal bulb. Although these anatomical differences appear obvious, the effects of fixation and specimen orientation frequently make the differences much more subtle.

Specimens of the crayfish Orconectes leptogonopodus and O. palmeri longimanus were collected from a site adjacent to U.S. Route 270, 2.3 miles east of Mount Ida, Montgomery County, Arkansas, by P. C. Holt on 23 June 1960. Four branchiobdellidans from this collection were mounted on a slide, USNM 100356 (= PCH 1090), and identified as Cambarincola serrata by Holt [all details come from the Worm General Catalogue under USNM 100356]. During the present investigation, these four specimens were remounted on separate slides, USNM 100356, USNM 170002, USNM 170003, USNM 170004, and identified as P. annulatus. This correction has maintained the reported distributional separation of the western C. serratus and the eastern Pterodrilus spp.

Discussion.—Unlike most of the species in the genus, *P. annulatus* can be grouped with *P. choritonamus* and *P. missouriensis*, as they also lack dorsal segmental appendages. The presence of a "differentiated" prostate gland in *P. annulatus* separates it from *P. missouriensis*, but provides a character in common with the rest of the species in the genus.

Holt (1968) reviewed the species of *Pter-odrilus* and discussed in great detail the "Primitive *Pterodrilus*," "Phylogeny of the Genus," and the "Places of Origin and Migration." Observations on *P. annulatus* have not resolved or clarified any of the concepts addressed by Holt, and so no value is seen in discussing these issues here.

#### **Emmended Species Descriptions**

# Pterodrilus cedrus Holt, 1968 Fig. 2A-B, 3E

*Specimens.*—Holotype and five paratypes (USNM 36464, = PCH 1396), and USNM 36465-36468.

Brief description.—Body terete 1.0 to 1.3 mm long, low dorsal ridges on segments 2 through 7 and prominent ridge across segment 8 with 5 finger-like projections (Fig. 3E), supernumerary muscles present; dorsal lip smooth; oral papillae present; dental formula 5/4; male genitalia extend upwards just beyond the level of the intestine; glandular atrium length about 1/3 segment diameter; prostate gland ends subterminal to glandular atrium, prostate [vacuolated] differentiated, ental bulb absent; muscular atrium length about two-thirds the glandular atrium; bursa oval, length about 1/3 segment diameter, penial sheath filling ental 1/2 of bursa; spermatheca length about 1.3 times segment diameter, ectal duct about 1/3 length, bulb tubular to oval, ental process absent.

*Variations.*—The prominence of the dorsal ridges on segments 2 though 7 depends on the degree of contraction and extent of curvature of the specimen. The shape of the spermathecal bulb varies greatly depending on the amount of sperm present in the lumen and the degree of displacement as it bends over the intestine (Figs. 2A and B).

Hosts.—Orconectes placidus (Hagen), O. rusticus subspecies, O. juvenilis (Hagen) and *Cambarus tenebrosus* Hay from Holt (1968:23).

*Distribution.*—Clay County, Tennessee from Holt (1968:21).

*Remarks.*—The holotype and three paratypes show five short finger-like projections which is contrary to the four reported by Holt (1968:21). The length of the muscular atrium is usually about  $\frac{2}{3}$  that of the spermiducal gland. However, in the holotype it is stretched to be of equal length. The spermatheca has an ectal duct  $\frac{1}{3}$  to  $\frac{1}{2}$  the total length of the organ, and not  $\frac{2}{3}$  as drawn by Holt (1968:22) in his figure 5a. An examination of the paratypes support the revised ratio.

# Pterodrilus choritonamus Holt, 1968 Fig. 31

The type description reports an ental process on the spermathecal bulb (Holt 1968: 27). However, an examination of the holotype (USNM 36471, = PCH 1395) and two paratype (USNM 35472a,b, = PCH 1395) specimens revealed that an ental process is absent. The ental end of the elongate spermathecal bulb is simply bent over the intestine and gives the appearance of a "process". The presence or absence of a spermathecal ental process is one of the criteria used in branchiobdellidan taxonomy (Holt 1968).

### Pterodrilus hobbsi Holt, 1968 Fig. 2C, 3F

Specimens.—Holotype (USNM 36486), five paratypes (USNM 36487), 194 other specimens (USNM 36488-36508), and (SRG 31–35).

*Brief description.*—body terete, 1.3 to 1.7 mm long, supernumerary muscles absent, dorsal ridge only across segment 8 with 5 finger-like projections (Fig. 3F); dorsal lip smooth; male genitalia extends to dorsad of segment; glandular atrium length about half segment diameter; prostate gland ends subterminal to glandular atrium, prostate [vacuolated] differentiated entirely or



Fig. 2. *Pterodrilus cedrus*: A, Lateral view of the reproductive organs in segments 5 and 6 in the holotype; B, Lateral view of the spermatheca in segment 5 in a paratype, scale bar =  $25 \mu m$ . *Pterodrilus hobbsi*: C, Lateral view of the reproductive systems in segments 5 and 6 in the holotype, showing the variation in the prostate gland with the double headed arrow, scale bar =  $25 \mu m$ .

ental <sup>2</sup>/<sub>3</sub>, ental bulb absent; muscular atrium length subequal to glandular atrium, bursa subspherical length <sup>1</sup>/<sub>4</sub> segment diameter, penial sheath filling ental <sup>1</sup>/<sub>3</sub> of bursa; spermatheca length about 0.8 to 1.1 times segment diameter, duct about <sup>1</sup>/<sub>3</sub> length, bulb ovoid, ental process absent.

Variations.-The dorsal finger-like pro-



Fig. 3. Lateral view of the dorsal surface of segments 1 to 8 in nine species of *Pterodrilus*: A, *P. distichus*; B, *P. simondsi*; C, *P. alcicornus*; D, *P. mexicanus*; E, *P. cedrus*; F, *P. hobbsi*; G, *P. annulatus*; H, *P. missouriensis*; I, *P. choritonamus* (A, B, and C were redrawn from Holt, 1968), scale bar = approximately 200  $\mu$ m. Each figure is arranged so that its respective segment 8, as labelled on the top row, is aligned in a column for ease of comparison. Abbreviations: 8, segment 8.

jections vary in length and spacing, therefore this is a dubious character for separating *P. hobbsi* from *P. cedrus*. The glandular atrium and prostate glands also differ slightly in relative length, and the extent of vacuolation in the prostate varies (Fig. 3, double-headed arrow). The size of the spermathecal bulb depends upon the amount of sperm product being stored and may be as small as <sup>1</sup>/<sub>4</sub> the size shown in Fig. 2C.

Hosts.—Cambarus tenebrosus Hay, C. longulus longirostris Faxon, C. parvoculus Hobbs & Shoup, C. longulus chasmodactylus James, C. robustus Girard, C. veteranus Faxon, C. friaufi Hobbs, C. extraneus Hagen, C. bartonii cavatus Hay, C. sciotensis Rhoades, C. distans Rhoades, C. bartonii bartonii (Fabricius), C. longulus longulus Girard, C. latimanus (LeConte), C. stratus Hay, Cambarus species, C. bartonii subspecies; Orconectes erichsonianus (Faxon), O. juvenilis (Hagen), O. rusticus forceps (Faxon), and Orconectes species from Holt (1968:20) and Orconectes placidens (Hagen, 1870).

Distribution.—This is summarized by Holt (1968) in his figure 9 and in a list of river systems and counties. For consistency, a distribution by counties has been compiled from the Worm General Catalogue of the material that Holt (1968:20) deposited in the museum: Lauderdale County, Alabama; Harlan County, Kentucky; Alleghany County, North Carolina; Anderson, Grainger, Hawkins, Johnson, Lawrence, McMinn, Overton, Roane, Unicoi, and Union counties, Tennessee; Bland, Carroll, Dickenson, Lee, Scott, and Washington counties, Virginia, and a new record from the Harpeth River, Williamson County, Tennessee (see type location of *P. annulatus*).

*Remarks.*—The description and drawings given here are based on the holotype, and differ from the type description figures given by Holt (1968:18). All of the material examined is consistent with the illustrations given (Figs. 2C, 3F).

# Key to species of *Pterodrilus* (Fig. 3)

la.	dorsal projections on any of segments
1	2-3 2
b.	ments 2–5 4
2a.	two finger-like projections on segments
	2–7 (Fig. 3A) <i>P. distichus</i>
b.	fan-like projections on segments 3-5.
	and 8 3
3a.	fan-like dorsal projections on segment
	2 (Fig. 3B)
b.	fan-like dorsal projections absent from
	segment 2 (Fig. 3C) P. alcicornus
4a	dorsal projections on prominent ridge
	of segment 8 5
h	dorsal projections absent from promi-
0.	nent ridge of segment 8 7
5a	four finger-like projections present (Fig
Ju.	3D) <i>P mexicanus</i>
h	five finger-like projections present 6
69.	ridges on segments 2-7 present (Fig
oa.	3F $P$ codrus
h	ridges on segments 2, 7 absent (Fig. 3F)
υ.	P hobbsi
7.	ridges on segments 2.7 high (Fig. 2C)
1a.	P annulatus
h	ridges on segments 2.7 low (Fig. 2H)
0.	D misses on segments 2-7 10W (Fig. 3H)
	ridere en este 2, 7 el cert (Eig. 21)
c.	ridges on segments 2–7 absent (Fig. 31)
	P choritonamus

Note: In 6b and 7c where ridges are absent, so are the supernumerary muscles. All species identifications made using keys should be confirmed with additional anatomical characters.

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